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John F. Hernandez Nopsa^a, P. Stephen Baenziger^b, Kent M. Eskridge^c, Kamaranga H. S. Peiris^d, Floyd E. Dowell^e, Steven D. Harris^a & Stephen N. Wegulo^a

^a Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE, 68583, USA

^b Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68583, USA

^c Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE, 68583, USA

^d Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS, 66506, USA

^e USDA ARS Center for Grain and Animal Health Research, Engineering and Wind Erosion Research Unit, Manhattan, KS, 66502, USA

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Epidemiology/Épidémiologie

Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions

JOHN F. HERNANDEZ NOPSA^{1,6}, P. STEPHEN BAENZIGER², KENT M. ESKRIDGE³,
KAMARANGA H. S. PEIRIS⁴, FLOYD E. DOWELL⁵, STEVEN D. HARRIS¹ AND STEPHEN N. WEGULO¹

¹Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

²Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

³Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

⁴Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506, USA

⁵USDA ARS Center for Grain and Animal Health Research, Engineering and Wind Erosion Research Unit, Manhattan, KS 66502, USA

⁶Current address: USDA ARS Forage Seed and Cereal Research Unit, 3450 SW Campus Way, Corvallis OR 97331, USA

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Abstract: Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe [sexual stage *Gibberella zeae* (Schwein.) Petch], is a destructive disease of small grain cereals. *Fusarium graminearum* produces the mycotoxin deoxynivalenol (DON), which accumulates in and lowers the value and quality of grain. Field experiments were conducted from 2007 to 2009 to determine if two winter wheat cultivars varying in FHB phenotype response differentially accumulated DON. Secondary objectives were to model the relationship between FHB severity and DON, determine if environment (= year) influenced DON accumulation in the two cultivars, and measure the percentage of *Fusarium*-damaged kernels (FDK) in the two cultivars. The cultivar ‘Harry’ with a moderately resistant FHB phenotype consistently accumulated more DON ($P \leq 0.0358$) than ‘2137’ with a susceptible phenotype. The relationship between FHB severity and DON was linear and positive for both cultivars in all three years ($0.32 \leq R^2 \leq 0.60$; $0.0053 \leq P \leq 0.1092$). Environment (= year) had a significant effect ($P < 0.0001$) on DON accumulation in both cultivars, and this effect was attributed to differences in rainfall amount and duration in the months of May and June. DON accumulation was highest in 2008 (average of 33.2 ppm in ‘Harry’ and 21.2 ppm in ‘2137’) when there was steady, above-average rainfall in May and June. FDK was highest in 2008 and was higher in ‘Harry’ (64%) than in ‘2137’ (46%). The results from this study suggest that a winter wheat cultivar with a moderately resistant FHB phenotype can be susceptible to FDK and DON accumulation. Based on these results, there is a need to standardize the criteria (FHB intensity, FDK, DON) for characterizing wheat cultivars as resistant or susceptible to FHB.

Key words: deoxynivalenol (DON), FHB index, *Fusarium*-damaged kernels (FDK), *Fusarium graminearum*, Fusarium head blight (FHB), winter wheat

Résumé: La fusariose de l’épi (FHB), ou gale, causée par *Fusarium graminearum* Schwabe [stade sexué *Gibberella zeae* (Schwein.) Petch], est une maladie qui ravage les petites céréales. *F. graminearum* produit la mycotoxine déoxynivalénol (DON) qui s’accumule dans les grains et qui en réduit la qualité et, par conséquent, la valeur. De 2007 à 2009, des expériences au champ ont été menées pour déterminer si deux cultivars de blé d’hiver, dont les phénotypes réagissaient différemment à la FHB, accumulaient différemment le DON. Les objectifs secondaires étaient de modéliser la relation entre la gravité de la FHB et le DON, de déterminer si l’environnement (= année) influençait l’accumulation de DON dans les deux cultivars et de mesurer le pourcentage de grains fusariés chez les deux cultivars. Le cultivar ‘Harry’, dont un phénotype est modérément résistant à la FHB, a invariablement accumulé plus de DON ($P \leq 0.0002$) que ‘2137’ dont le phénotype est

Correspondence to: Stephen N. Wegulo. E-mail: swegulo2@unl.edu

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sensible. La relation entre la gravité de la FHB et le DON était linéaire et positive pour les deux cultivars durant les trois années ($0.32 \leq R^2 \leq 0.60$; $0.0053 \leq P \leq 0.1092$). L'environnement (= année) avait un effet significatif ($P \leq 0.0001$) sur l'accumulation de DON chez les deux cultivars. Cet effet était attribué aux différences dans la quantité et la durée des précipitations durant les mois de mai et juin. L'accumulation de DON était la plus importante en 2008 (moyenne de 33.2 ppm chez 'Harry' et de 21.2 chez '2137') lorsque les pluies étaient constantes et au-dessus de la moyenne en mai et juin. Il y avait plus de grains fusariés en 2008, et ce, davantage chez 'Harry' (64 %) que chez '2137' (46 %). Les résultats de cette étude suggèrent qu'un cultivar de blé d'hiver dont le phénotype est modérément résistant à la FHB peut afficher une certaine teneur en grains fusariés et en DON. En se basant sur ces résultats, il y a lieu de standardiser les critères (intensité de la FHB, grains fusariés, DON) permettant de caractériser les cultivars en tant que résistants ou sensibles à la FHB.

Mots clés: blé d'hiver, déoxynivalénol (DON), fusariose de l'épi (FHB), *Fusarium graminearum*, grains fusariés, indice de fusariose

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [sexual stage *Gibberella zeae* (Schwein.) Petch] is an economically important disease of wheat (*Triticum aestivum* L.) and other small grain cereals in the United States and other parts of the world. FHB causes premature bleaching of spikelets on wheat spikes (Dill-Macky, 2010). Bleached spikelets are sterile or contain shrivelled, discoloured kernels commonly referred to as *Fusarium*-damaged kernels (FDK). FHB results in yield loss and poor grain quality (Parry *et al.*, 1995; McMullen *et al.*, 1997; Dexter & Nowicki, 2003). In addition, *F. graminearum* produces the toxin deoxynivalenol (DON) which accumulates in and lowers the quality of grain (Parry *et al.*, 1995; McMullen *et al.*, 1997). Since 2007, epidemics of FHB have occurred in the Central Great Plains, causing significant economic losses in hard winter wheat in the states of Kansas, Nebraska and South Dakota (USWBSI, 2007–2011). Research is needed to identify cultivars with resistance to both FHB and DON. Such cultivars can be used as germplasm in breeding programmes aimed at developing new cultivars with improved resistance to FHB.

Previous studies have shown that FHB-susceptible wheat cultivars generally accumulate more DON than resistant cultivars. In North Carolina, Cowger *et al.* (2009) showed that when post-anthesis mist was applied or not applied to *F. graminearum*-inoculated soft red winter wheat, the susceptible cultivar 'USG 3592' accumulated more DON than moderately resistant cultivars. In Hungary, Mesterházy *et al.* (1999, 2003) observed more DON accumulation in susceptible than in resistant winter wheat cultivars. In Germany, Koch *et al.* (2006) similarly observed that a highly susceptible winter wheat cultivar accumulated more DON than a moderately resistant cultivar. Previous research also demonstrated, however, that DON accumulated in FHB-resistant small grain genotypes. Arseniuk *et al.* (1999) found that both FHB-resistant and -susceptible wheat, triticale and rye

genotypes accumulated DON in kernels. Teich *et al.* (1987) found that a soft winter wheat cultivar with a moderate incidence of FHB accumulated less DON than a cultivar with a low FHB incidence. Gosman *et al.* (2005) showed that resistance derived from an FHB-resistant winter wheat cultivar appeared to have a greater effect on colonization of grain by the fungus than on DON accumulation in the grain.

The association between FHB intensity and DON accumulation has been studied extensively. Paul *et al.* (2005) performed a quantitative review of research findings to determine if there was a consistent and significant relationship between FHB intensity and DON concentration in harvested wheat grain. Using meta-analysis, they analysed 163 published and unpublished studies and found more than 65% of correlation coefficients (r) between measures of FHB intensity and DON concentration to be > 0.50 . The relationship between diseased spike severity and DON concentration was linear and positive, with a mean r of 0.53.

In the current study, we report the accumulation of higher concentrations of DON in a winter wheat cultivar with a moderately resistant FHB phenotype compared with a cultivar with a susceptible phenotype. We also report a positive, linear relationship between FHB severity and DON concentration in grain for both cultivars, a significant effect of environment (= year) on DON accumulation, and a higher percentage of FDK in the moderately resistant compared to the susceptible cultivar.

Results from field experiments in 2007 indicated that the cultivar 'Harry' had a moderately resistant FHB phenotype, but accumulated DON concentrations comparable to or higher than those in the cultivar '2137' with a susceptible phenotype. Field studies were initiated to confirm these observations. The objectives were to (1) determine if the winter wheat cultivar 'Harry' with a moderately resistant FHB phenotype accumulated more DON than the FHB-susceptible cultivar '2137'; (2) model the relationship between FHB severity and DON concentration in the

two winter wheat cultivars in objective 1; (3) determine the effect of environment (= year) on DON accumulation in the two winter wheat cultivars in objective 1; and (4) determine if the two cultivars in Objective 1 differed in the percentage of *Fusarium*-damaged kernels (FDK).

Materials and methods

Weather

Weather data were provided by the High Plains Regional Climate Center's Automated Weather Data Network (High Plains Regional Climate Center, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA).

Phenotypic reaction to *Fusarium* head blight

Data used to characterize the phenotypic reaction of cultivars 'Harry' and '2137' were obtained from two studies conducted at the University of Nebraska Agricultural Research and Development Center near Mead, NE. The first study (Wegulo *et al.*, 2011) was conducted to determine the effects of integrating cultivar resistance and fungicide application on FHB and DON accumulation. The second study (unpublished) was conducted to determine the effect of planting date and inoculation timing on FHB and DON accumulation. In the second study, three winter wheat cultivars ('Harry', '2137', and 'Jagalene') were planted on two planting dates in the fall of 2006 (2007 cropping season) and 2007 (2008 cropping season). The cultivars were chosen to represent those commonly grown in Nebraska during the previous three years. The planting dates were 5 October (first planting date; '2137') and 9 October (first planting date; 'Harry' and 'Jagalene') and 27 October (second planting date; all three cultivars) in 2006 and 27 October (first planting date; all three cultivars) and 10 November (second planting date; all three cultivars) in 2007. Seed was planted with a small plot drill at a seeding rate of 72 kg ha⁻¹ in 2006 and 101 kg ha⁻¹ in 2007. Plot size was 1.5 m × 9.1 m in the 2007 cropping season and 2.0 m × 9.1 m in the 2008 cropping season. The experimental design was a split-split-plot in randomized complete blocks with three replications with planting date as the main plot, cultivar as the subplot, and inoculation treatment as the sub-subplot. The inoculation treatments consisted of no inoculation and inoculation at early anthesis and mid anthesis (Zadoks growth stages 60 and 65, respectively; Zadoks *et al.*, 1974). Plots were inoculated with a spore suspension (1 × 10⁵ spores mL⁻¹) of an isolate of *F. graminearum* previously obtained from an infected wheat kernel

from a Nebraska wheat field. A hand-pumped back-pack sprayer was used to inoculate plots. Natural inoculum was also present in the field. Index was assessed 21 days after inoculation. Index in the non-inoculated treatment was assessed 21 days after the early anthesis inoculation treatment.

Current study

The study was conducted at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Two winter wheat cultivars were planted on 5 October 2006 ('Harry') and 9 October 2006 ('2137') following soybean (*Glycine max* L. (Merr.)) harvest. The same two winter wheat cultivars were planted on 27 October 2007 and 3 October 2008 following corn (*Zea mays* L.) harvest. 'Harry' is a late maturing hard red winter wheat cultivar (Baenziger *et al.*, 2004) with a moderately resistant FHB phenotype (Wegulo *et al.*, 2011). '2137' is a hard red winter wheat cultivar (Sears *et al.*, 1996) susceptible to FHB (Wegulo *et al.*, 2011). The cultivars were chosen to represent those commonly grown in Nebraska during the previous three years. Seed was planted with a small plot drill at a seeding rate of 72 kg ha⁻¹ in 2006, 101 kg ha⁻¹ in 2007 and 80 kg ha⁻¹ in 2008. Plot size was 3.1 m × 9.2 m in 2007, 2.0 m × 9.2 m in 2008, and 1.5 m × 3.3 m in 2009. Cultivars were arranged in a randomized complete block design with three replications.

Inoculation

Field plots were inoculated as described above at mid anthesis with spores of the same isolate of *F. graminearum* at 1 × 10⁵ spores mL⁻¹. There also was natural inoculum in the field. Mid anthesis was considered to be the day on which 50% of the heads of a given cultivar had extruded anthers. Thus, cultivars were inoculated on different dates within a season ranging from 28 May to 4 June. Plots were not irrigated.

Disease assessment and harvesting

Disease assessment was done 21 days after inoculation in all three years. Twenty spikes were tagged representing each of the following FHB severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50% severity in 2007 and 2009. In 2008, there were insufficient numbers of spikes with low disease severity; therefore, spikes were tagged in the following severity categories: 20, 25, 30, 35, 40, 45, 50, 70 and 90%. Tagging facilitated

the identification, separation and hand harvesting of each spike in each disease severity category. Plot index was assessed on 600 spikes in each plot (20 spikes in each of 30 arbitrarily selected locations in each plot). Hand harvesting of tagged spikes was done when grain moisture content dropped below 15%. The rest of the wheat was harvested with a small plot combine.

FDK and DON analysis

The percentage of *Fusarium*-damaged kernels (FDK) in grain from each plot was determined at the USDA ARS Center for Grain and Animal Health Research, Engineering and Wind Erosion Research Unit, Manhattan, KS using a single-kernel near-infrared (SKNIR) system (Dowell *et al.*, 2006). The system sorted FDK based on a calibration developed from a partial least squares regression (Dowell *et al.*, 2006). Visually, these kernels were shrivelled and/or chalky white or pink in colour. Measurement of FDK by the SKNIR system has been validated (Wegulo & Dowell, 2008). Grain samples from tagged spikes in each FHB severity category were ground to flour (Laboratory Construction Co., Kansas City, MO. Model 256 grinder), and sent to the North Dakota Veterinary Diagnostic Laboratory, North Dakota State University, Fargo ND for DON analysis using gas chromatography with electron capture detection (GC/ECD) (Tacke & Casper, 1996).

Data analysis

Treatment effects or their interactions were considered significant at $P \leq 0.05$. For purposes of the current study, index data (for characterization of the phenotypic reaction of cultivars to FHB) from the study to determine the effects of planting date and inoculation timing on FHB were analysed with the general linear models procedure (GLM, SAS version 9.2; SAS Institute, Cary, NC) according to a randomized complete block experimental design. Planting dates in each year were combined based on homogeneity of error variances determined from the F-ratio test (Gomez & Gomez, 1984). Index was calculated as $[\text{incidence (\%)} \times \text{severity (\%)}]/100$.

In the current study, the GLM procedure of SAS version 9.2 (SAS Institute, Cary, NC) was used to do analysis of covariance (ANCOVA) (Gomez & Gomez, 1984; Steel *et al.*, 1997) on DON measured in grain from increasing severity categories with (i) DON as the variable of primary interest (dependent variable), FHB severity as a covariate, and cultivar as a categorical factor and (ii) DON as the variable of primary interest, FHB severity as a covariate,

and year as a categorical factor. To test for differences between regression slopes relating DON concentration to FHB severity for the two cultivars, cultivar \times severity and year \times severity interactions were included in the model statement in the analysis of covariance. The F-test was used to compare (between cultivars) DON least squares means generated by ANCOVA. Plot data (index, FDK and DON) were subjected to analysis of variance using the GLM procedure of SAS. Fisher's least significant difference (LSD) test (Gomez & Gomez, 1984; Steel *et al.*, 1997) was used to compare pairs of treatment means. Linear regression analysis (Gomez & Gomez, 1984; Steel *et al.*, 1997) was used to model the relationship between FHB severity and DON concentration.

Results

Weather

In southeast Nebraska, winter wheat flowering normally occurs in late May to early June and harvesting occurs in late June to mid July. The 30-year average precipitation at Mead from 1981 to 2010 was 101 mm in May and 105 mm in June (High Plains Regional Climate Center, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA). In 2007, there was above average rainfall in May followed by a relatively dry June (Table 1, Fig. 1). In 2008, there was steady, above average rainfall during both May and June. In 2009, May was relatively dry. Rainfall started in late May and continued through June, with average precipitation for the month of June (Table 1, Fig. 1). Relative humidity (RH) and temperature for the two months were similar in all three years except May 2009 when maximum RH was below 90% (Table 1). Therefore, weather conditions during the 3-week period before wheat flowering (anthesis) and through the flowering period in late May to early June favoured FHB infections in 2007 and 2008. In 2009, favourable weather for infections did not occur until the end of the first week in June (Fig. 1).

Phenotypic reaction to *Fusarium* head blight

In 2007 in the early anthesis inoculation treatment, FHB index in '2137' was more than twice that in 'Harry' and the index in 'Jagalene' was nearly twice that in 'Harry' with both '2137' and 'Jagalene' having significantly higher index than 'Harry' (Table 2). In the mid anthesis inoculation treatment, index was higher than in the early anthesis inoculation and non-inoculated treatments in all three cultivars. Index in '2137' and 'Jagalene' was significantly higher than in 'Harry' by 52 and 71%, respectively.

Table 1. Weather parameters at Mead, Nebraska, USA where experiments were conducted in 2007–2009 to investigate the accumulation of deoxynivalenol (DON) in two winter wheat cultivars: ‘Harry’ (moderately resistant to Fusarium head blight (FHB), high DON accumulator) and ‘2137’ (susceptible to FHB).

Year	Month	Total rain (mm) ^a	Average RH (%)	RH range	Average min temp (°C)	Average max temp (°C)	Average temp (°C)
2007	May	160	68.1	46.3 – 91.1	12.4	25.0	18.7
	June	40	70.2	43.4 – 90.6	15.4	28.1	21.8
2008	May	137	65.6	35.5 – 93.8	8.4	22.2	15.3
	June	234	70.0	48.0 – 90.9	15.4	28.4	21.9
2009	May	25	58.4	37.7 – 85.8	9.5	24.5	17.0
	June	107	75.5	40.1 – 94.8	15.2	27.1	21.1

^aThe 30-year (1981–2010) average rainfall at Mead was 101 mm in May and 105 mm in June (High Plains Regional Climate Center, School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA).

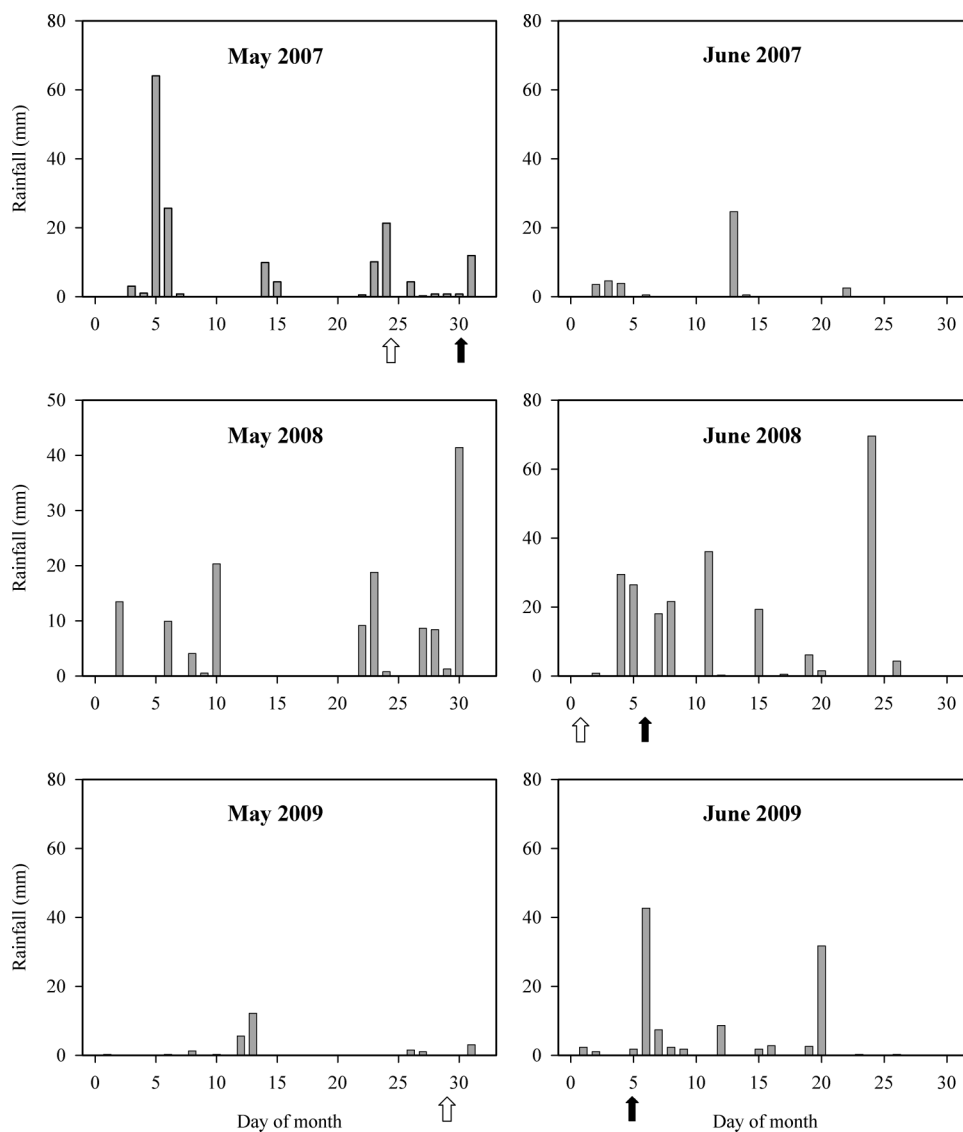


Fig. 1. Rainfall in May and June of 2007–2009 at Mead, Nebraska, USA where experiments were conducted to determine the phenotypic reaction of three winter wheat cultivars (‘2137’, ‘Harry’ and ‘Jagalene’) to Fusarium head blight and the differential accumulation of deoxynivalenol in two winter wheat cultivars (‘2137’ and ‘Harry’). The white arrow indicates the beginning of anthesis in ‘2137’ and ‘Jagalene’ whereas the black arrow indicates the beginning of anthesis in ‘Harry’.

Table 2. Fusarium head blight (FHB) index (%)^a in three winter wheat cultivars subjected to three inoculation treatments (early anthesis, mid anthesis and non-inoculated) in an experiment to determine the phenotypic reaction (moderately resistant or susceptible) of the three cultivars to FHB in 2007 and 2008.

Year	Cultivar	Inoculation treatment and index (%)		
		Early anthesis	Mid anthesis	Non-inoculated
2007	Harry	19.6 c ^b	36.6 b	22.1 b
	2137	44.5 a	55.6 a	41.9 a
	Jagalene	35.0 b	62.5 a	38.8 a
2008	Harry	5.8 b	14.0 c	7.2 b
	2137	18.6 a	31.5 b	18.0 a
	Jagalene	24.4 a	42.8 a	22.5 a

^aIndex was assessed 21 days after inoculation. Index in the non-inoculated treatment was assessed 21 days after the early anthesis inoculation treatment. Each index value is a mean of two planting dates and three replications per cultivar (n = 6).

^bMeans within a column in a year followed by the same letter are not significantly different according to the least significant difference test at P = 0.05.

This result (significantly lower index in 'Harry' than in '2137' and 'Jagalene') was consistent in all inoculation treatments in both years, with 2137 having an index two to three times that of Harry in 2008 (Table 2). A similar trend was observed in a study to determine the effects of integrating cultivar resistance and fungicide application on FHB and DON (Wegulo *et al.*, 2011), except that in that study, 'Harry' had a higher index than '2137' and 'Jagalene' in 2009 because rainfall coincided with anthesis in 'Harry' whereas it was dry during anthesis in '2137' and 'Jagalene' (Fig. 1). Even then, the index in 'Harry' in non-fungicide-treated plots was only 10.3% (Wegulo *et al.*, 2011), within a range that characterizes the cultivar as phenotypically moderately resistant.

Differences between '2137' and 'Harry' in plot index, FDK and DON concentration

In 2007, FHB plot index was significantly higher in 2137 than in 'Harry' (Table 3). The two cultivars did not significantly differ in FDK and DON (Table 3). In 2008, FDK in 'Harry' was significantly higher than in '2137'. Although index was higher in '2137' than in 'Harry' and the reverse was true for DON, these differences were not significant at P = 0.05. In 2009, the two cultivars did not significantly differ in index, FDK or DON (Table 3).

Differences in DON concentration in tagged spikes between '2137' and 'Harry' and among years

The effect of cultivar on DON accumulation was significant (P ≤ 0.05) in all three years (Table 4), with

Table 3. Plot Fusarium head blight (FHB) index, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) concentration in two winter wheat cultivars, 2007–2009.

Year	Cultivar	Index (%)	FDK (%)	DON (ppm)
2007	Harry	16.7 b ^a	19.7 a	1.1 a
	2137	45.7 a	12.7 a	1.5 a
2008	Harry	12.0 a	64.0 a	11.9 a
	2137	19.7 a	46.0 b	9.3 a
2009	Harry	6.5 a	30.4 a	1.7 a
	2137	0.4 a	29.8 a	1.0 a

^aMeans within a column in a year followed by the same letter are not significantly different according to the least significant difference test at P = 0.05.

'Harry' consistently accumulating more DON than '2137' (Fig. 2). Similarly, the effect of year on DON accumulation was highly significant (P < 0.0001), with both cultivars accumulating more DON in 2008 than in 2007 and 2009 (Table 5, Fig. 2).

Relationship between FHB severity and DON concentration in tagged spikes in '2137' and 'Harry'

There was a positive linear relationship between FHB severity and DON in all three years regardless of cultivar (Figs 3, 4; Tables 4, 5). A test of differences between regression slopes relating FHB severity and DON showed no significant differences between the two cultivars in two of the three years (Table 4) and no significant differences among years in the cultivar 'Harry' (Table 5). However, in the cultivar '2137', regression slopes relating FHB severity and DON were different among years (Table 5, Fig. 4).

Discussion

In this study, the cultivar 'Harry' with a moderately resistant FHB phenotype consistently accumulated more DON than the cultivar '2137' with a susceptible phenotype in spikes with FHB symptoms under field conditions. This observation is contrary to some previously published studies which showed that susceptible winter wheat cultivars accumulated more DON than moderately resistant cultivars (Miller *et al.*, 1985; Mesterházy *et al.*, 1999; Mesterházy *et al.*, 2003; Cowger *et al.*, 2009). The reason for the higher DON accumulation in the moderately FHB-resistant 'Harry' compared with the FHB-susceptible '2137' is not known. Some previous studies similarly reported the accumulation of larger amounts of DON in moderately FHB-resistant compared with FHB-susceptible genotypes. Teich *et al.* (1987) found a soft

Table 4. Analysis of covariance with cultivar as a categorical factor, deoxynivalenol (DON) as a dependent variable and FHB severity as a covariate. Data were obtained from field experiments conducted at Mead, Nebraska, USA in 2007–2009 to investigate the accumulation of DON in symptomatic spikes tagged in increasing FHB severity categories in two winter wheat cultivars ('2137' and 'Harry').

Source of variation	d.f. ^a	2007		2008		2009	
		MS ^b	P > F	MS	P > F	MS	P > F
Severity _{Linear} (S _L) ^c	1	3.37	0.0001	348.9	0.0021	189.2	0.0001
Cultivar (C) ^d	1	61.5	0.0043	1947.6	0.0358	167.8	0.0203
S _L x C ^e	1	7.7	0.0008	55.5	0.1910	1.5	0.6929

^aDegrees of freedom.

^bMean square.

^cTest for linear effect of severity.

^dThe F-test for comparing cultivar least squares means was based on pooling replication sums of squares with error sums of squares.

^eTest for different cultivar slopes.

Table 5. Analysis of covariance with year as a categorical factor, deoxynivalenol (DON) as a dependent variable and FHB severity as a covariate. Data were obtained from experiments conducted at Mead, Nebraska, USA in 2007–2009 to investigate the accumulation of DON in symptomatic spikes tagged in increasing FHB severity categories in two winter wheat cultivars ('2137' and 'Harry').

Source of variation	d.f. ^a	Harry		2137	
		MS ^b	P > F	MS	P > F
Severity _{Linear} (S _L) ^c	1	202.9	0.0005	326.2	<0.0001
Year	2	7594.5	<0.0001	3331.1	<0.0001
S _L x Y ^d	2	9.2	0.7330	120.3	<0.0001

^aDegrees of freedom.

^bMean square.

^cTest for linear effect of severity.

^dTest for different slopes across years.

winter wheat line with a low incidence of FHB to accumulate more DON than a line with a moderate FHB incidence. Arseniuk *et al.* (1999) showed that both FHB-resistant and -susceptible wheat, triticale and rye genotypes accumulated DON in kernels. They concluded that regulation of DON accumulation may be independent of FHB reaction. Mesterházy *et al.* (1999) found that some wheat genotypes with low FHB severity had high DON contamination and vice versa.

Several mechanisms of resistance to DON accumulation in wheat and other small grains have been proposed. Research by Miller *et al.* (1985) suggested that resistant cultivars have factors that prevent synthesis and/or promote degradation of DON. Snijders & Krechting (1992) showed that in a resistant wheat line, translocation of DON from chaff to grain was inhibited and this resulted in little fungal colonization of the grain. Research is needed to determine whether the late maturing characteristic of 'Harry' may be a contributory factor to the high DON

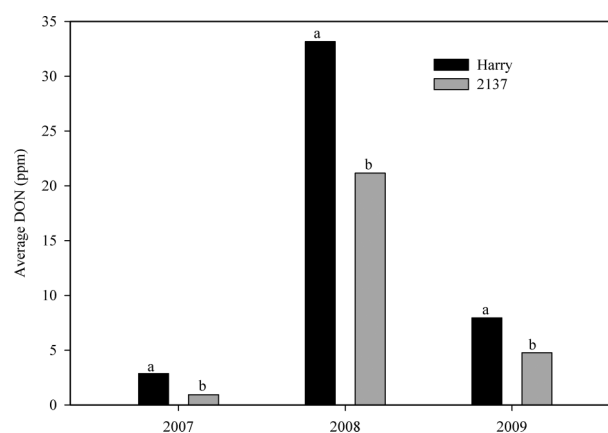


Fig. 2. Average deoxynivalenol (DON) concentration in grain from tagged spikes in experiments conducted at Mead, Nebraska, USA in 2007–2009 to investigate the accumulation of DON in two winter wheat cultivars: 'Harry' (moderately resistant to Fusarium head blight (FHB), high DON accumulator) and '2137' (susceptible to FHB). Spikes were tagged according to severity categories ranging from 0 to 50% in 2007 and 2009 and according to severity categories ranging from 20 to 90% in 2008. Means (bars) with the same letter within a year are not significantly different at $P = 0.05$ according to the t-test.

accumulation in this cultivar. Research by Cowger & Arrellano (2010) led them to conclude that late infection by *F. graminearum* is an important factor leading to low FDK and high DON in soft winter wheat. Further investigation is warranted to determine why the cultivar 'Harry' is susceptible to DON accumulation despite a moderately resistant FHB phenotype.

In addition to susceptibility to DON accumulation, plot data in the current study and in a previously published study (Wegulo *et al.*, 2011) showed that the cultivar 'Harry' also had FDK levels higher than or comparable to those in the FHB-susceptible '2137', implying that Harry is also susceptible to FDK. Because FDK and

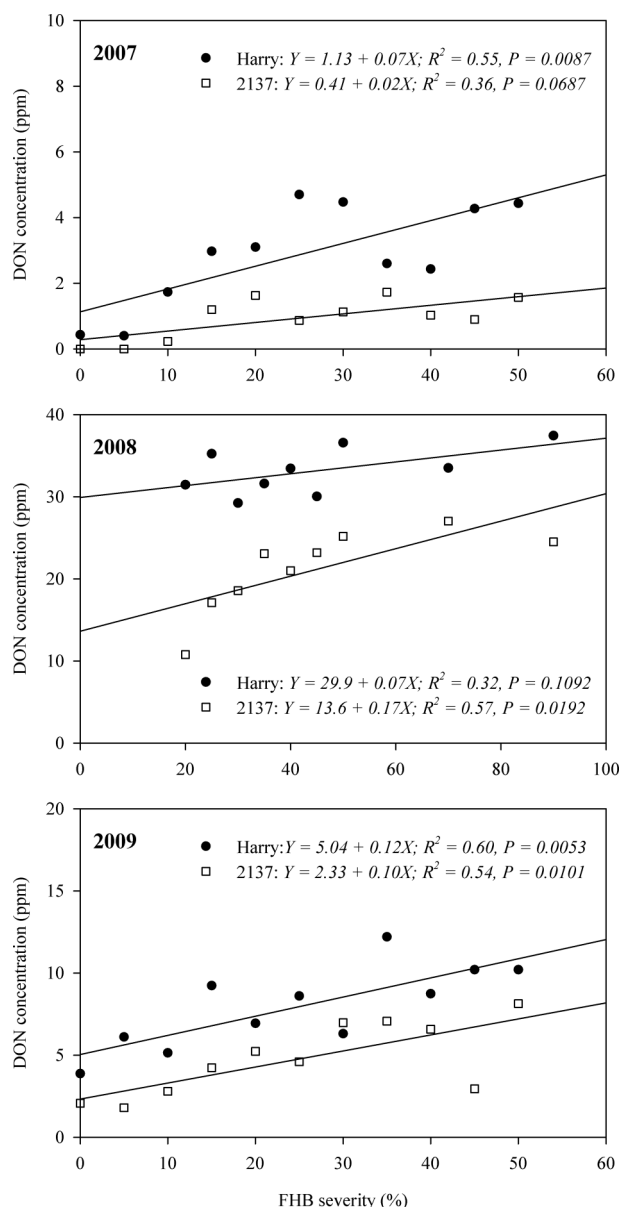


Fig. 3. Regression of deoxynivalenol (DON) concentration on Fusarium head blight (FHB) severity by cultivar within year. Data were obtained from experiments conducted at Mead, Nebraska, USA in 2007–2009 to investigate the accumulation of DON in two winter wheat cultivars: ‘Harry’ [moderately resistant to Fusarium head blight (FHB), high DON accumulator] and ‘2137’ (susceptible to FHB).

DON are positively correlated (Paul *et al.*, 2005; Wegulo *et al.*, 2011), this observation suggests that the higher DON accumulation in ‘Harry’ may be due in part to the cultivar’s susceptibility to FDK. The susceptibility to FDK and DON accumulation in a phenotypically FHB-resistant cultivar warrants standardization of the criteria (FHB intensity, FDK, DON) used to characterize wheat cultivars as resistant or susceptible to FHB. A cultivar

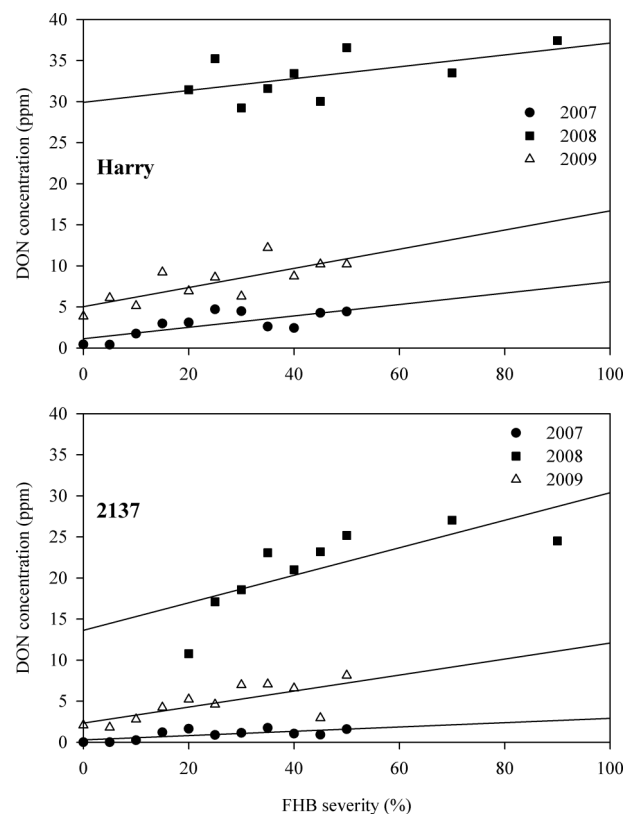


Fig. 4. Regression of deoxynivalenol (DON) concentration on Fusarium head blight (FHB) severity by year within cultivar. Data were obtained from experiments conducted at Mead, Nebraska, USA in 2007–2009 to investigate the accumulation of DON in two winter wheat cultivars: ‘Harry’ [moderately resistant to Fusarium head blight (FHB), high DON accumulator] and ‘2137’ (susceptible to FHB). Regression equations: ‘Harry’, 2007: $Y = 1.13 + 0.07X$; $R^2 = 0.55$, $P = 0.0087$; ‘Harry’, 2008: $Y = 29.9 + 0.07X$; $R^2 = 0.32$, $P = 0.1092$; ‘Harry’, 2009: $Y = 5.04 + 0.12X$; $R^2 = 0.60$, $P = 0.0053$; ‘2137’, 2007: $Y = 0.41 + 0.02X$; $R^2 = 0.36$, $P = 0.0687$; ‘2137’, 2008: $Y = 13.6 + 0.17X$; $R^2 = 0.57$, $P = 0.0192$; ‘2137’, 2009: $Y = 2.33 + 0.10X$; $R^2 = 0.54$, $P = 0.0101$.

can have three separate characterizations depending on whether it is resistant or susceptible to FHB on the spikes, FDK, or DON; or it can be characterized based on some index that takes into account all three criteria.

Analysis of plot data in the current study showed that ‘Harry’ had a higher FHB index than ‘2137’ in 2007 and 2008, but not in 2009. This result, which is consistent with a similar result in a separate experiment conducted in the same location (Wegulo *et al.*, 2011) was due to the timing of rainfall with anthesis, the period when most FHB infections occur (Dill-Macky, 2010). In 2007 and 2008, rainfall coincided with anthesis in both cultivars. However, in 2009, it was dry during anthesis in ‘2137’ and rainfall coincided with the onset of anthesis in ‘Harry’ about six days after anthesis in ‘2137’. Analysis of plot data also

showed that there were no significant differences between the two cultivars in FDK in 2007 and 2009 and in DON concentration in all three years, with low levels of DON in 2007 and 2009. The low plot DON levels were due in part to mixing of healthy and *F. graminearum*-infected grain. In addition, loss of FDK which are lighter than healthy kernels during combine-harvesting likely contributed to the low DON levels (the grain in tagged, symptomatic spikes was hand-harvested). This observation implies that DON and FDK can be more accurately measured in grain from symptomatic spikes that are hand-harvested compared with bulked, machine-harvested grain.

Environment (= year) had a significant effect on DON accumulation in both cultivars. DON accumulation in 2008 was much higher than in 2007 and 2009. This was most likely due to the rainfall pattern, duration and amount in May and June. In May 2007, infection by *F. graminearum* was favoured by heavy rainfall before and during anthesis. However, disease development was slowed down by dry weather in June, which limited DON accumulation. In 2009, the reverse was true, with May being relatively dry followed by a moderate amount of rainfall in June. Therefore, infection by *F. graminearum* was limited due to the lack of adequate moisture before and during flowering in 2009, and this similarly resulted in the accumulation of low amounts of DON. In contrast, there was steady, above average rainfall in both May and June in 2008, and this resulted in high DON accumulation. Research by Cowger & Arrellano (2010) suggested that longer moisture durations, as occurred in the current study in 2008, favour the spread of late infections within spikes, resulting in higher percentages of FDK and higher DON levels. The observation in this study that yearly differences in rainfall pattern, duration and amount accounted for the significant effect of environment (= year) on DON accumulation is supported by the findings of Kriss *et al.* (2010). Using a window-pane analysis, they showed that moisture- or wetness-related variables including relative humidity and precipitation were associated with yearly fluctuations in Fusarium head blight intensity.

The relationship between FHB severity and DON was linear and positive in both cultivars and in all three years. Previous studies have similarly shown a linear and positive relationship between FHB intensity and DON. Wegulo *et al.* (2011) found a consistent, positive correlation between FHB index and DON in five field experiments conducted over a period of three years. Using meta-analysis, Paul *et al.* (2005) analysed 163 studies and found an overall positive, linear relationship between FHB intensity (index, severity or incidence) and DON. Knowledge about the relationship between FHB intensity and DON can be used to estimate the amount of DON to

expect in grain based on observed disease intensity levels during the growing season. This can in turn enable producers to make timely, informed marketing decisions.

Results showed that in the low DON years (2007 and 2009), FDK in 'Harry' was not significantly different from that in '2137'. However, in 2008 (high DON year) FDK in both cultivars was higher than in 2007 and 2009 and FDK in 'Harry' was significantly higher than in '2137'. The overall higher FDK in 2008 (high DON year) than in 2007 and 2009 (low DON years) is in agreement with the findings of previous studies which reported a positive correlation between FDK and DON (Paul *et al.*, 2005; Veitch *et al.*, 2008; Wegulo *et al.*, 2011). The higher FDK in 'Harry' compared with '2137' in 2008 is consistent with the findings of a separate study in which Wegulo *et al.* (2011) found FDK in 'Harry' in fungicide-unsprayed field plots to be significantly higher than that in '2137' and was expected since FDK has been shown to be positively correlated with DON (Paul *et al.*, 2005). FDK, like FHB intensity, can be a reliable predictor of DON levels in grain.

This study demonstrated that a winter wheat cultivar with a moderately resistant FHB phenotype accumulated more DON than a susceptible cultivar. This finding indicates that although in general FHB-susceptible cultivars accumulate more DON than moderately resistant ones, some moderately resistant cultivars can be susceptible to relatively high levels of DON accumulation. Resistance to both FHB and DON accumulation should be considered by producers when selecting the cultivars to plant, and by breeders when developing new cultivars.

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